

## PAPOVAVIRUS T ANTIGENS

POLYOMA: A consensus is emerging on the role of the various T antigens in viral oncogenesis/transformation (part of the game here is to keep the distinctions between these two phenomena in view). T (big T) is dispensible: DNA fragments without the totality of T are tumorigenic, cells infected with - but not transformed by - hrt mutants contain lots of T (but not functional t or middle T), non-producer transformants often (usually, inevitably?) do not even express T, presumably because of the configuration of the provirus; revertants retain T; microinjections of DNA fragments have shown T to be dispensible for transformation in culture. Middle T seem essential in all regards; if defective, neither transformation nor tumorigenesis occurs. Little T (t) remains in the shadows: it may be required only in certain contexts: it may ~~be~~ mimic cell growth factors already present in some cells or some contexts. In view of the preceeding, the location and known prlp properties of the antigens are of interest. As well established, T is nuclear, a DNA binding protein, required for the initiation of viral DNA synthesis, and capable of inducing cellular DNA synthesis; the last talent may be irrelevant~~xxxx~~ to tumorigenesis, in contrast of older views. Middle T is on or in the plasma membrane and may (note conditional sense of the word) be a protein kinase - sound familiar? t is cytoplasmic; nothing else is known of its properties or location.

SV40: Chaos reigns. t is either essential or dispensible, or essential, depending on the iv investigator; T is either essential or dispensible, depending on the investigator. The confusion may arise from the different contexts of testing - growth factors,

type of cell infected, ~~xxxx~~ animal used. ~~SV40~~ SV40 transformend human cells aren't even tumorigenic in nude mice. Efforts to force the SV40 system into a full analogy with Py have failed, to date. Of course, there is a candidate middle T (dubbed ~~xxxx~~ "phantom T" by local talent) proposed o by Berg on the basis of a new splice his troops have found in a late version of early message (clear?). The splice creates a new reading frame and, accroding to the DNA sequence, would generate a hydrophobic protein with a hydrophobic carboxy-terminal domain, not unlike that of middle T of Py (and pp60<sup>src</sup> ...). The phantom would be vi the same size as conventional T and therefore could have been overlooked in previous analyses. Berg has not found the protein, to date. TSTA has been identified; it is a version of T (or phantom T?) in the membrane, ~~xxx~~ surface labelled only if the intact cells are first fixed with formaldehyde (this is a relatively new perception that may change current views on the significance of surface labelling and force repitition of older experiments).

#### Bona Fide Tumor Antigen

The mysterious 53K proteins found ~~xx~~ by various hands in cells transformed by SV40 (but not in lytic infections) has come into better focu focus and proven to a more general phenomenon than previously appreciated. It appears to be a polymorphic protein, variant from species to species, barely detectable (if at all) in normal ac cells (except for undifferentiated EC cells), induced by SV40 transformation and, it turns, out , by other forms of oncogeneisis, as well: Jay has found t what is proabaly the a same protein in a host of m neoplastic cells by using antibodies raised against various MCA-induced tumors. Locales include: SV , Py, ASV transformatns, leukeima cells, MSV transformants

spontaneously transformed cells, etc. It also appears in normal cells during early G<sub>1</sub>. The protein is phosphorylated and can be precipitated either directly by antisera or by virtue of its apparent ability to bind to T antigen. It is apparently a nuclear protein, but nothing else is known of its function. Pollack says that expression of 53K protein is a correlate of anchorage-independent growth, but can uncouple it from all individual forms of T antigen expression by the use of mutants - confusing. The protein is made from a 17S cell mRNA and when synthesized in vitro in concert with T does not bind to the latter - curious. Deletions in T overproduce T and pp53. The form of T that binds pp53 is hyperphosphorylated and a minor fraction (ca. 5%) of the total T.

Carroll has found another such ~~40~~ protein - ca. 48K.. It is also nuclear, but is neither as polymorphic nor as ubiquitous as the pp53 - found only in SV-transformed cells. It appears to be a cellular protein whose expression is related to expression of A gene.

The proteins secreted by normal and transformed proteins reported previously by Hynes lab were reviewed. Some are phosphorylated, none are related to any of the above Tm antigens, induced cellular proteins, src or proto-src. Little new, in other words.

Kasamatsu reported two induced proteins, 14 and 18K, that appear as antigens ~~in the~~ juxtaposed to the centriole in cells transformed by SV40 (and perhaps other viruses - data not secure). These could explain the "perinuclear" location of pp60<sup>src</sup> claimed by Rohrschneider, since Kasamatsu found antibodies against the proteins in random tumor antisera, but not in normal sera.

## THE EARLY REGION OF ADENOVIRUS

Presentations focused on the transcription, splicing and regulation of this region and, in the words of no less than Phil Sharp, "bewildering", and certainly provided no information bearing directly on the main topic of the meeting. Transfection has been carried to the extreme in this system: 0-5 map units induces aneuploid, immortal cells, but not transformation in the full sense; 0-10 map units does the whole thing. A number of proteins encoded in these regions have been identified, including the T antigen defined and studied by our own ADL, but the role of any or all of these in trans transformation remains a conundrum (in my view).

### ASV src.

No genuine surprises ~~(are)~~ (other than that some of the vaunted opposition has not gotten too far). Erikson has kinase activity soluble and pure enough (it appears) to claim the following: autophosphorylation, cyclic independence (confirmation of previous suspicions) and phosphorylation of several substrates, including actin, desmin, casein, and some other cytoskeletal proteins not identified in my brief conversation with him. Kinase prepared from ts infected cells appears to have ts phenotype, using these new substrates. The kinase of numerous ts src mutants recovers activity on temp shift down, then decays in about 6-12 hours, in absence of new protein synthesis; this reverses the old view that src expression cannot generally reverse in the absence of protein synthesis. The src product ~~xxxxxxx~~ is "rephosphorylated" during the recovery. Pastan has used a filter assay to study some properties of the kinase in the immune complex form. He reports: deoxy and ribo triphosphate both used as donors - this is unusual; ribodiphosphates inhibit, as do deoxydi's, with complex dose response curves; preincubation of the complex with ATP "inactivates" the kinase (or is it merely phosphoryating

all of the available sites on the substrate?); and TLCK inactivates the kinase. He then posed two, to my mind contradictory ideas: the properties of the immune complex reaction suggest that the protein is not a kinase, and the results with TLCK (which has long been known to effect a reversion of the transformed phenotype) implicate the kinase activity in transformation... You figure it out.

Work on localization has been hot and heavy. Rohrshniedern now has it perinuclear (but see above), diffusely cytoplasmic, and membranous - all based on IF. Goldberg has it principally in the membrane, based on data quite analogous to those obtained here by our dauntless Anglo-American axis. He also claimed to get surface staining by IF after (but not before) formaldehyde fixation (again, see above). Pastan ~~xxxx~~ (actually, Mark Willingham) has used ferritin-tagged Ab to locate pp60<sup>src</sup> (or its antigenic activity) beneath or on the cytoplasmic surface of the PM, especially in ruffles and at cell junctions. The pictures were quite nice.

The vole revertants have now produced two stories: tumorigenic revertants (yes, you read correctly) - reversion apparent only in the cytoskeleton and LETS - retain an apparently active pp60<sup>src</sup>, although marginal data suggested that the B p V8 fragment might be larger than customary; complete revertants have defective pp60 - kinase activity way down - presumably a mutation. in src.

Wyke described a series of newly isolated non-conditional tds which he believes (but has yet to prove biochemically) are deletions scattered throughout src - If he is correct, these could be quite useful.

The pp60 of recovered ASV has been analysed in some detail. The Hanafusa strains appear to really contain both amino and carboxy termini derived from the parental td, but as much as 80% of the total is derived from proto-src. The reconstituted src genes of derived from chicken and quail appear quite similar by fingerprinting

of oligos.

~~xxxxMAYxxxx~~

MSV src. Still lagging, but mapping by transfection with cloned DNA has ~~been~~ better defined the coding region, at least for Mo strain: maximum complexity, ca 800 bp. This conforms to the emerging consensus that the protein product in several strains is ca. 20-28K, phosphorylated ~~and~~ Some strains of MSV may encode a polyprotein as described for various defective leukemia viruses - this is not yet resolved; for example, there was no new data on the identity of the virion kinase that Sen has claimed to be a src product, cleaved out of a polyprotein precursor. Efforts to definitively identify the src mRNA of MSV have not yet succeeded, at least according to reports at the meeting. ~~xxxxxx~~ Nothing was heard from the Salk axis, whose rather intriguing data we saw some weeks ago in L.X.

#### DEFECTIVE LEUKEMIA VIRUSES

A lot of action! In the bird world: Cooper has succeeded with low efficiency transfection by both MC29 and AEV - has recovered virus from transformed mammalian cells - and has performed a second transfection in sequence. No blotting or protein work has yet been accomplished on the transfectants. The European brigade reports the following genome sizes: AMV, 6200; OK10, 8100 (other results carried no surprises). Probes specific for one of MC, AEV and AMV detect cellular homologues, Nonproducers of AMV may contain both a polyprotein (P130) and Pr76, a finding which I call the sg genome sizing into question, unless I completely misunderstood the story. Grag has both a ts and a non-conditional mutants of AEV; both affect only the erythrocyte transformation by the virus, not the fibroblast transformation. The td appears to be a deletion because the P76 is a tad

and the deletion allegedly mapping by peptides in onc portion, smaller than usual. Eiasenman has repa repeated the unpublished experiments of local talent, cla cleaving the P110 (or P120 in some cases in his hands; I did not understand this) with the p15 of ASV/ALV and using the cleaved products to define ~~max~~ the onc portion of the protein. A surprising result using this approach: ~~the P75 of AEV~~ the mysterious viral P100 found in uninfected RBCs is composed of gag elements like those found in the P75 of E AEV plus non-viral peptides. As we knew, the onc portion of the cleaved polyproteins are hihg highly phosphorylated.

Similar - and, in some instances, mor advanced - stories are emerging for the ~~max~~ mammalian viruses. Abelson p P120 is in the membrane, with onc portion at the cell surface, perhaps gag portion at the cytoplasmic surface. It has a phosphorylated onc portion and may (may) have a protein kinase ~~activity~~ active on itself but not on Ig. As found here for MC, ~~max~~ nonproducers tranformed by Ab contain a single viral mRNA - the genome, thus further implicating the polyprotein in transformation. The Abelsonm map: Mo helper 1320 bp - onc, 3600 bp - helper 730 bp. A proto sequence has been found in mammalian DNA with a specific probe, and antisera (from regressor mice, with activity g against onc) have detected a P150 in normal <sup>bone marrow & spleen</sup> thymus (in <sup>at least partial</sup> accord with Risser previous work using cytotoxic Ab); ) 2/12/79 this endogenous protein has onc determinants only, not gag or env. Risser extended his work on differentiation antigens related to onc of Ab and Friend virus - whether his antigens and the proteins precipitated by similar sera are identical has yet to be determined. Fv-2 locau (which specifiies susceptibility to Friend oncogenesis) apparnelty controls the expression of the antigen in normal cells. Positive tissues for Fr include fetal liver, bone marrow and spleen. This is gratifying, because it is a different spectrum wh than that found with Abelson. (and thus sustatins the argument that the normal cell protein and

the related viral gene r product are differentiation factors of some sort). Mak presented related work, using a probe specific for SFFV: homologous RNA found in certain tissues (spleen, BM), amounts under control of Fv-2 and <sup>three</sup> other loci (<sup>SL</sup>W<sub>1</sub> and H2).

Other polyproteins~~xxx~~ with putative onc: MCF (<sup>r</sup>suprise), 98K, processed by p15 of AMV; FeSV ~~and~~ (also alleged to have autokinase)11 and RadLV (100K - tenuous). The only candidate for transforming protein of SFFV at present in is a gp55 found at the cell surface and <sup>homologous</sup> analogous <sup>with</sup> to the gp<sub>90</sub> of MCF (an alleged contributor to the oncogenicity of SFFV).

The FOCMA story ~~&~~ remains unclear. The protein is allegedly One version of the antigen is alleged to be the product of the onc portion of a fused gene in FeSV, yet the same or related antigen occurs on the surface of cat leukemia cells (but not on the surface of untransformed cells infected with FeLV). A single antigen, p70, is found on the sru surface of the luek leukemia cl cell, two (pp85 and p70) on the surface of FeSV transfromred cell. None of these are glycosylated.

#### MURINE THYMOTROPIC LEUKEMIA

Focus here is on MCF viruses, but there is no new evidence that might implicate these viruse in tumorigenesis more effectively. Rowe has bred the Akv-1,2 loci into congeni background of NFS vi mice which carry no recognized eco endogenous virus and has recovered MCF from these beasts. They are different than the origiala isoaltes from AKR and other conventional strains - as defined by fingerprinting of the RNA (Hopkins), biological properties, etc. The individual isolates are ~~xxxxxx~~ nevertheless distinctive, prompting the suggestion that each has a differe, but relat e, xenotropic paretn. The alleged Xeno parent for MCF have yet to be identified other than as antigens that emerge in the developing h thymus with appropriate kinetics. chronology. The One set of MCFs h share a "novel"

oligo at or near the 3' end of the genome. This finding exercised (naively, in my view) a clique who have hit upon the seized upon the "novel promoter" mechanism as a potential explanation for enigmatic forms of leukemogenesis.

The role of glycosylated gag precursor proteins on cell surfaces remains uncertain. They occur as differentiation antigens in normal mice (the GCSA, for example). An unidentified soul in the audience announced the identification of similar molecule on surface of ASV infected fibroblasts and AMV transformed myeloblasts; no data. In a possibly related or unrelated finding, Famulari reported nice studies showing that gPr env on the surface of murine leukemia surface ~~xxxxxx~~ was a specific finding correlated with the uniquely slow processing of the precursor in leukemia cells.

#### GROWTH FACTORS

Todaro no longer argues ~~that the GF he and DeLarco identified~~ (secreted from MSV-transformed cells) is a src product. ~~Some properties of SGF (sarcom GR):~~ 9-10K, not EGF, although g binds to EGF receptors; sustains anchorage independent growth by normal cells. Idea: viral TF induces production of such factors and these are major element in the TF phenotype. Todaro and Sen also suggested that the virion kinase reported by Sen is not only the src product, but itself a growth factor when applied to cells and will sustain anchorage-independent growth. Sounded quite provisional.